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Feeding Behavior in *Aiptasia tagetes* (Duchassaing and Michelotti) Planulae: A Plausible Mechanism for Zooxanthellae Infection of Aposymbiotic Planktotrophic Planulae

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ABSTRACT. – **The common West Indian sea anemone, *Aiptasia tagetes* (Duchassaing and Michelotti), is a natural host for the symbiotic dinoflagellate, *Symbiodinium* sp., in which direct transmission of zooxanthellae from the host to the eggs does not occur. Under normal ambient conditions *A. tagetes* was found to be dioecious and oviparous. Feeding experiments on the ovoid ciliated planulae of *A. tagetes* were conducted. Planulae readily ingested *Uca* sp. claw meat, *Artemia* sp. flakes and zooxanthellae. Zooxanthellae originating from egested balls of zooxanthellae from the host, from freshly isolated cells of the host, as well as those naturally occurring in the seawater system were all accumulated by the planulae. Planulae were maintained for a maximum of 35 days (longest to survive) in culture and zooxanthellae were retained by the planulae for the entire period. It is unknown whether the planulae became infected by the zooxanthellae.**

RESUMEN. – ***Aiptasia tagetes* (Duchassaing and Michelotti), una anémona común de las Antillas Occidentales, es un hospedero natural del dinoflagelado simbiótico, *Symbiodinium* sp., donde no ocurre transmission directs de zooxantela del hospedero a huevo. *A. tagetes* es dioica y ovípara bajo condiciones ambientales normales. Se llevaron a cabo experimentos de alimentación de la planula ciliada y ovoide de *A. tagetes*. Las planulas ingirieron con rapidez carne de palanca de *Uca* sp., hojuelas de *Artemia* sp. y zooxantelas. Las planulas acumularon zooxantelas provenientes de bolas de zooxantelas expulsadas por el hospedero, de células aisladas del hospedero, y también provenientes del sistema de agua salada. Las plánulas se mantuvieron en cultivo por un máximo de 35 días (las que sobrevivieron el máximo) durante los cuáles las plánulas retuvieron las zooxantelas. Se desconoce si las plánulas fueron infectadas por las zooxantelas.**

INTRODUCTION

There has been much interest in cnidarian-zooxanthellae relationships principally in the areas of taxonomy of the algal symbiont (Freudenthal, 1962; Kevin et al., 1969; Taylor, 1974; Blank and Trench, 1985a, b, 1986), physiology (Muscatine, 1967, 1971; Trench, 1971a-c), regulation of algal numbers in the host (Taylor, 1969; Steele, 1976), and specificity in the symbioses (Schoenberg and Trench, 1976; Coney and Trench, 1983). Effects of the zooxanthellae on adult hosts have also been studied (Kinzie and Chee, 1979; Fitt, 1985a). Selectivity and recognition of the algae are integrated aspects which play a central role in establishing the symbiotic association (Kinzie, 1974; Trench et al., 1981; Fitt, 1984, 1985b).

The symbiotic dinoflagellate, *Symbiodinium* sp. (Blank and Trench, 1986), is harbored by a large number of marine cnidarians (Taylor, 1974; Trench, 1979). Many of these cnidarians do not directly transmit the zooxanthellae to their offspring (Fitt and Trench, 1981; Fitt, 1984) indicating that the algal symbiont must be acquired independently. It has been suggested that chemical cues, "intermediate" hosts, and random contact of the potential host and zooxanthellae aid in the infection of aposymbiotic cnidarian hosts (Muscatine, 1973; Kinzie, 1974; Trench, 1979; Trench et al., 1981; Fitt, 1984, 1985b). However, there are few direct observations of ingestion of zooxanthellae by larvae. One such report was that of Krupp (1983) in which planulae of the coral, *Fungia scutaria* Lamarck, became infected following ingestion of zooxanthellae swarmers.

Aiptasia tagetes (Duchassaing and Michelotti), a common sea anemone which in-

habits reefs and mangrove roots in the West Indies, is a natural host of *Symbiodinium* sp. (Schoenberg and Trench, 1976). The present paper describes qualitative observations of feeding behavior by planulae of *A. tagetes.* In addition, ingestion of zooxanthellae by the aposymbiotic planulae is discussed.

Materials and Methods

Adult specimens of *Aiptasia tagetes* were collected from mangrove roots in La Parguera near the La Parguera field station of the Department of Marine Sciences, University of Puerto Rico several days before spawning. Male and female pairs, maintained in 10 cm or 18 cm fingerbowls immersed in an ambient temperature seawater bath on an outdoor wet table under natural sunlight, spawned during July and December 1979; April, May, July, September and October 1981; and in March 1982. Spawn in the fingerbowls were first observed as ova, embryos or planulae; none of these contained zooxanthellae. The aposymbiotic condition was verified by conventional light microscopy at 1000×.

Planulae were removed from the fingerbowls containing the parent anemones and reared separately in fingerbowls under the conditions detailed above. Number of planulae per fingerbowl varied from a few to one or two thousand planulae. These planulae were examined daily for general appearance and behavior. For experimental treatments the seawater was either unfiltered, filtered. through 0.45 µm millipore filters, or filtered through 35 µm Nytex plankton netting. Another group of planulae was kept with the parents or other adult *Aiptasia tagetes* in 0.45 µm millipore filtered seawater.

Five qualitative feeding regimes were employed in which planulae were fed:

1. Freshly isolated zooxanthellae. These cells were obtained by macerating an *Aiptasia tagetes* polyp in a glass tissue grinder and centrifuging the homogenate to yield a pellet of zooxanthellae which was resuspended in seawater.
2. Dried and pulverized *Uca* sp. (fiddler crab) claw meat. The *Uca* claw meat was initially dyed with either methylene blue or carmine to facilitate feeding observations.
3. Pulverized commercially prepared *Artemia* sp. flakes.
4. The unicellular alga, *Isochrysis* sp. (Tahitian).
5. Unfed.

The seawater was changed daily in treatments 2 through 4; the seawater of planulae in treatment 1 and the unfed planulae were always changed more frequently than every three days but sometimes daily.

Unstained squash preparations made from small pieces of gonad removed through the mouth or cinclides of field collected *Aiptasia tagetes* polyps yielded information on sex and gonadal maturity. Additional information was gained from examination of histological sections of polyps prepared using standard histological techniques (Riggs, 1982).

Results

Under normal environmental conditions at La Parguera, Puerto Rico, *Aiptasia tagetes* was found to be dioecious and oviparous. Planulae were obtained from night-time spawnings during the months mentioned above. The planulae were ovoid, ciliated, measured up to 109 x 138 µm and possessed an apical tuft which measured about half the body length (Fig. 1A). The planulae were judged to be aposymbiotic if zooxanthellae were not detectable by conventional microscopy.

In most cases, planulae remained free swimming and initially seemed strongly attracted to light. Planulae were commonly observed to swim along the tentacles into and out of the mouths of adults, and when planulae were present in high concentrations, they were observed to remain inside parents and non-parents for days. Spawned ova were also seen inside the hollow tentacles. Planulae did not demonstrate avoidance behavior in the presence of non-parent *Aiptasia tagetes.* After two or three days planulae were frequently observed resting on the bottom of the fingerbowls mouth down, swimming into the water column when disturbed. While not measured, nei-

Discussion

Feeding in *Aiptasia tagetes* planulae was investigated during attempts to complete its life cycle. It was thought that nutrition and/or acquisition of zooxanthellae would be important in survival since they are assumed to be planktotrophic larvae (Chia, 1976). This idea led to several incidental observations.

Aiptasia tagetes planulae were observed temporarily attached mouth down, a behavior thought to represent feeding in planulae of *Aiptasia* sp. (Widersten, 1968) and *Metridium dianthus* (Ellis) (Gemmill, 1920). The *A. tagetes* planulae readily ingested pulverized crab meat as well as commercially prepared *Artemia* sp. flakes, Zooxanthellae cells were also readily ingested, but *Isochrysis* sp. cells were rejected. Siebert (1974) described the ingestion of 1 μm plastic spheres and mucus strands but rejection of *Isochrysis* sp. algal cells (10 μm diameter) by planulae of *Anthopleura xanthogrammica* (Brandt). As a result of his observations, Siebert concluded that selection of ingested material by these planulae may be based on size of material with 1 μm spheres preferred to 10 μm algal cells. This is not consistent, however, with feeding of *Aiptasia tagetes* planulae, which are smaller than planulae of *Anthopleura xanthogrammica. Isochrysis* sp. cells fed to *Aiptasia tagetes* planulae measured ca. 6 μm, the zooxanthellae cells were 10-12 μm in diameter and the crab meat particles much larger. Therefore, selection of small size particles does not seem to determine food preference of *Aiptasia tagetes* planulae. Palatability of food is probably more important as planulae of both *Aiptasia tagetes* and *Anthopleura xanthogrammica* rejected the alga, *Isochrysis* sp.

Until recently the methods by which infection of cnidarian hosts by zooxanthellae took place was uncertain. Several aposymbiotic invertebrate offspring are infected as a result of zooxanthellae cells entering the host via the mouth. These include gorgonian polyps, *Pseudopterogorgia bipinnata* (Verrill) (Kinzie, 1974), scyphistomae of *Cassiopea xamachana* Bigelow (Trench et al., 1981; Coney and Trench, 1983; Fitt, 1984), and veligers of *Tridacna squamosa* Lamarck (Fitt and Trench, 1981).

The likelihood of infection resulting from random contact may seem unlikely; however, the observation that a single isolated planula came in contact with and ingested at least 20 free "swimming" zooxanthellae cells (although this could have resulted from mitosis of one or more cells) shows that this can occur. The fact that embryos develop to the planula stage within 24 h, increases the probability of encounter with zooxanthellae during their early development. Chemical attractants may also play a role in such encounters (Fitt, 1985b). Both motile and non-motile algae may readily be ingested by mobile aposymbiotic larvae, though motile zooxanthellae may be more likely to be encountered in the water column (Fitt, 1984). *Aiptasia tagetes* is frequently found in large numbers inhabiting mangrove roots, and sponges on coral reefs, potentially providing a major source of the appropriate infecting zooxanthellae. Another available source of zooxanthellae may be the motile stage of zooxanthellae released from the feces of reef fishes and nudibranchs that feed on cnidarians (Parker, 1984). *Aiptasia* species are not limited to infection by their own strain of *Symbiodinium* sp. However, there are differences in rates of zooxanthellae cell proliferation (Schoenberg and Trench, 1976) and growth of the host (Kinzie and Chee, 1979) with type of zooxanthellae. Intermediate hosts may provide the only natural source of symbiotic algae associated with animal tissue. Fitt (1984) showed that zooxanthellae transported via intermediate hosts are capable of infecting scyphistomae of *Cassiopea xamachana,* a necessary requisite for strobilation. Ingestion of fecal pellets from cnidarian predators resulted in the infection of aposymbiotic *Aiptasia pulchella* Carlgren adults (Parker, 1984). High numbers of viable zooxanthellae and semi-digested animal protein are contained in the feces of such predators (Parker, 1984). Trench et al. (1981) showed that the presence of host animal tissue enhanced the uptake of freshly isolated zooxanthellae by scyphistomae of *C. xamachana.*

Healthy *Symbiodiniurn* sp. cells are present in the pellets periodically egested from *Aiptasia tagetes* (Steele, 1976) and are presumably a suitable source of algae for the aposymbiotic planulae. Since the planulae feeding on the egested zooxanthellae were maintained with adult anemones it is difficult to determine the length of time the zooxanthellae were retained by these planulae.

Following acquisition of zooxanthellae from the environment, phagocytosis is the initial process involved in establishing the symbiotic association in aposymbiotic coelenterate offspring (Coney and Trench, 1983). Even if some zooxanthellae were phagocytosed by endodermal cells of the *Aiptasia tagetes* planulae it is possible that they did not persist and were lost. Trench et al. (1981) found that when scyphistomae of *Cassiopea xamachana* phagocytosed heterologous strains of zooxanthellae, some of the strains did not persist and were eliminated, probably by exocytosis, within 24 to 72 h. The question of whether phagocytosed zooxanthellae are digested remains unanswered (Trench, 1979; Trench et al., 1981). In this study, freshly isolated zooxanthellae from *A. tagetes* were retained by the isolated planulae for at least 31 days after feeding was stopped.

Though not quantified, the results suggest that the planulae can recognize and select material to be ingested. Whether animal protein is selected for, digested or egested remains unknown but it is obviously a preferred food. It is also not known whether the planulae of *Aiptasia tagetes* that retained zooxanthellae cells phagocytosed, digested or egested any of the cells.

Krupp (1983) first documented the subsequent infection of planulae derived from aposymbiotic ova, although he did not actually observe the ingestion of zooxanthellae. Though infection of *Aiptasia tagetes* planulae was not definitively demonstrated in this study, the ingestion of zooxanthellae cells through the mouth was observed, verifying at least one mechanism of acquisition of the algae in aposymbiotic planktotrophic planulae.

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